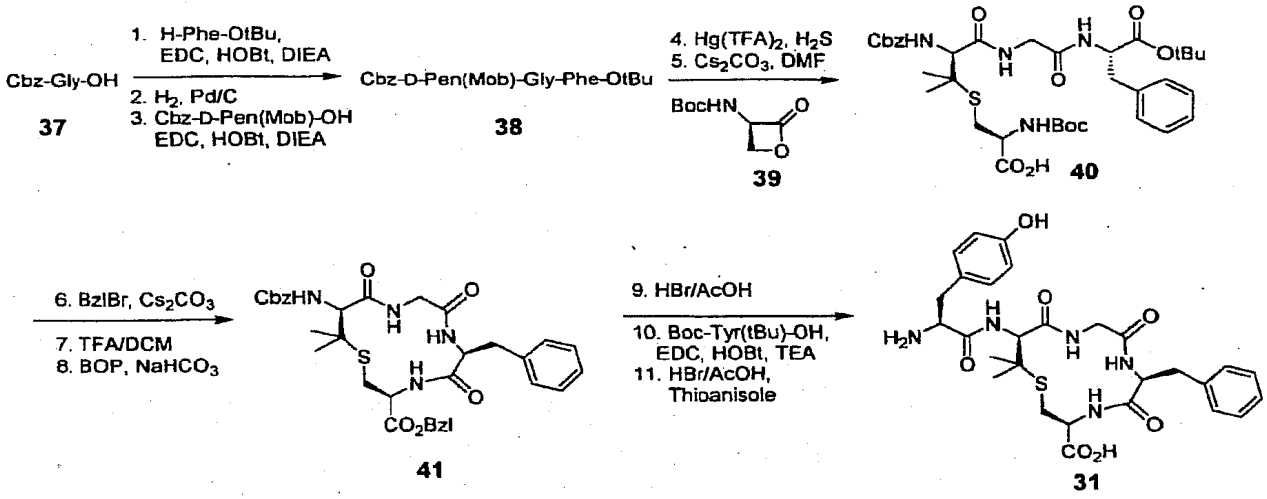


SCHEME 11 Synthesis of Tyr–c[D-Val_L–Gly–Phe-D–Ala_L]–OH **31** in solution.

group was coupled to Boc–Tyr(tBu)–OH with EDC/HOBt. The cyclic pentapeptide was deprotected by 30% HBr/AcOH in the presence of thioanisole providing the final structure **31**, which was purified by reverse phase HPLC (RP-HPLC).

There are several disadvantages in this scheme including poor overall yield through 11 steps in solution, competitive formation of thioester in the reaction of the β-lactone with free thiols, particularly with cysteine,[63] and epimerizations of α-carbons at position 2 or position 5 during overall synthesis in solution. Although synthesis of Tyr–c[D-Val_L–Gly–Phe–D/L-Ala_L]–OH **31/32** was successful, further attempts to obtain Tyr–c[D-Ala_L–Gly–Phe–D/L-Ala_L]–OH **33/34** failed because of the problems noted above. Therefore, a new approach for the derivatization of target analogs was required.

Synthesis of Tyr–c[D-Val_L–Gly–Phe-D–Ala_L]–OH 31 on Solid Phase

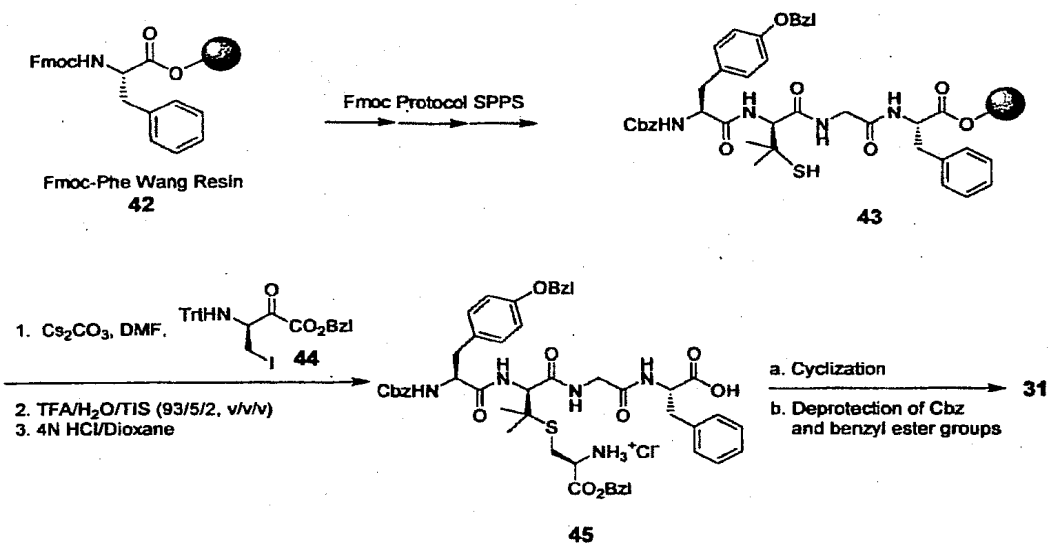
Recently, the synthesis of suitably protected lanthionines for solid phase peptide synthesis was reported by Ménez (Scheme 10, route C).[66] The *N*-trityl-3-iodoalanine derivatives were used to overcome the loss of chirality by epimerization of the α center or via β elimination. However, a significant amount of undesired aziridine was obtained (up to 35%). As a result, we applied this method to solid phase reactions in which the required purification step is not necessary.

The target peptide **31** was synthesized via preparation of the linear peptide **45** on solid support and cyclization in solution (Scheme 12).[48,49] The key step in the synthesis is the introduction of the lanthionine linkage by the reaction of *N*-trityl-D-3-iodoalanine benzyl ester **44** with the tetrapeptide resin **43** in the presence of Cs_2CO_3 in DMF. The other lanthionine analogs, including Tyr-c[D-Val_L-Gly-Phe-D-Ala_L]-OH **32** and Tyr-c[D-Ala_L-Gly-Phe-D/L-Ala_L]-OH **33/34**, were also prepared without epimerization. This synthetic approach represents a more efficient route for preparing lanthionine enkephalin analogs than synthesis in solution. (The details of the synthesis and the biological activities will be published elsewhere.)

Methylamine-Bridged Cyclic Enkephalin Analogs

Synthesis of amine-bridged cyclic enkephalin analogs represents another intriguing approach in the design of cyclic peptides. There are several advantages in using an amine as a bridge atom. These molecules can be obtained with great structural diversity based on commercially available or readily synthesized amines. They incorporate a site for protonation under physiological conditions and the trivalent amine can serve as a "handle" by which to functionalize the bridge without disturbing the pharmacophores. These modifications have the potential to modulate biological activity or biodistribution of the target structures.[50]

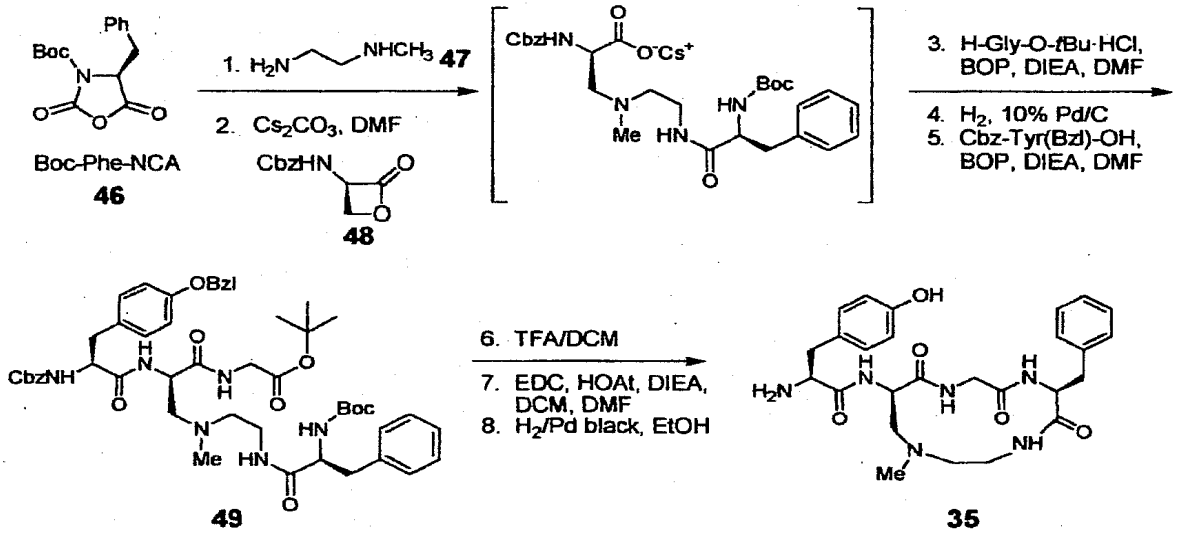
We have reported the synthesis and biological activity of a 13-membered methylamine-bridged enkephalin [MABE(I), **35**][50] and a 14-membered methylamine-bridged enkephalin [MABE(II), **36**].[48] The previous synthetic strategy for the synthesis of lanthionines by ring opening of serine β-lactone was applied in the synthesis of MABE(I) in solution. The key step involves the regioselective ring opening of



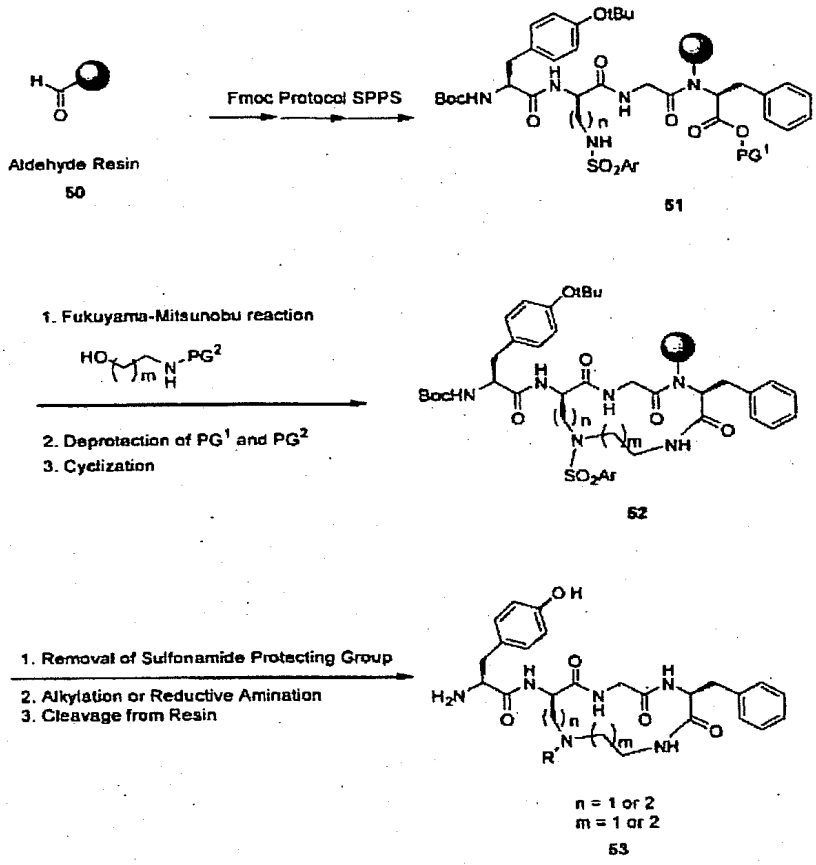
SCHEME 12 Synthesis of Tyr–c[D-Val_L–Gly–Phe–D-Ala_L]–OH **31** on solid phase.

the *N*-Cbz-D-serine β-lactone **48** by the secondary amine, which was obtained by coupling of *N*-methylethylenediamine **47** with Boc–Phe–NCA **46** with Cs_2CO_3 in DMF. The synthesis of MABE(I) **35** was completed by the route shown in Scheme 13. The synthesis involves 8 total steps and an overall yield of 7.4%. The MABE(II) **36** was obtained by preparation of the linear peptide on solid phase followed by cyclization in solution.[48] (Full paper about MABE(II) and related analogs will be published elsewhere.)

The potency of these methylamine-bridged enkephalin analogs makes them promising new lead compounds in our research for enkephalin analogs with selective activities. Since the synthetic route for the synthesis of MABE(I) described above was not adequate for the construction of libraries of amine-bridged enkephalin analogs which are needed for the study of structure–activity relationships, we recently developed a combinatorial synthetic route for the derivatizations of MABE(I) and MABE(II) (Scheme



SCHEME 13 Synthesis of MABE(I) **35** in solution.



SCHEME 14 On-resin synthesis of amine-bridged enkephalin analogs.

14). (The details of the synthesis will be reported elsewhere.)

An aldehyde resin **50**[67–72] was employed to prepare the linear peptide backbone **51** having a Boc group and an orthogonal protecting group 1 (PG^1) as terminal protecting groups. The Fukuyama–Mitsunobu reaction[70–73] was utilized to prepare sulfonamide-protected secondary amines which become the various tertiary amine bridges for the target analogs. The Fukuyama–Mitsunobu reaction of aminoalcohol (with the amine protected by a suitable protecting group 2, PG^2) with resin **51** provides an efficient route for the synthesis of secondary amines **52** protected by arylsulfonamide group ($ArSO_2$-) which is removable under mild conditions. Therefore, we were able to carry out cyclization, deprotection of the sulfonamide protecting group, and alkylation on the resin. The target peptidomimetic structures **53** were obtained after cleavage of the resulting cyclic molecules from the resin.

Thus, we have created a general route for the solid phase synthesis of diverse amine-bridged opioid structures.

Conclusions

We have devised routes for the synthesis of heteroatom-bridged opioids. These structures represent attractive candidates for the design of potent and selective analogs. In addition, a wide variety of amine bridged molecules can be prepared by combinatorial techniques that will permit us to study structure–bioactivity relationships in a comprehensive manner.

CONCLUDING PERSPECTIVES

The above discussion of new reagents, reactions, and target opioids summarizes some of our current research, and is indicative of directions for our future

efforts. The core of the research could not have been undertaken without the pioneering discoveries of Bruce Merrifield. It is truly an honor for us to participate in the Symposium and report on our synthetic activities.

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3-Hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazene

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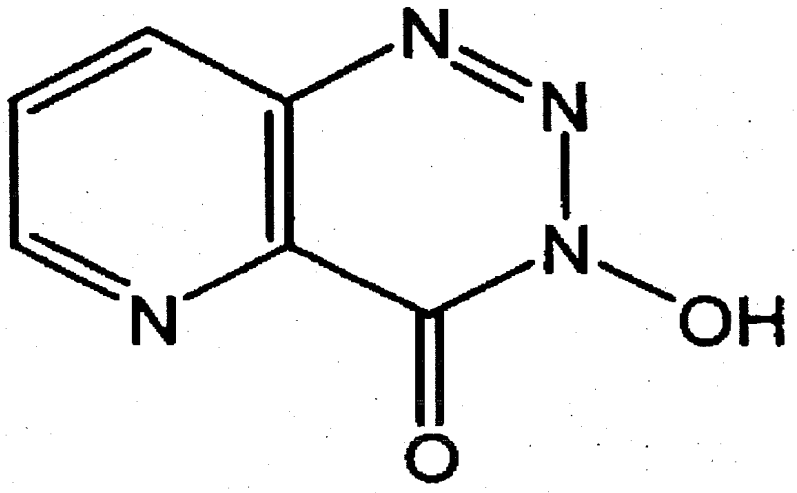

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JOC*Article*

3-Hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazene†

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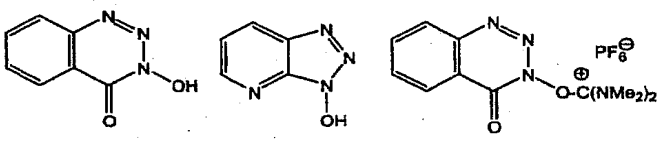
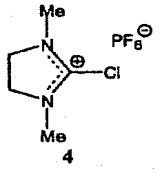
The known but long-neglected compound HODhat was shown to be in certain situations a useful peptide coupling additive. Uronium and phosphonium salts with HODhat built into the system were also useful stand-alone coupling reagents. Comparisons with related additives and coupling reagents showed that the new systems were sometimes more and sometimes less effective than previously described systems in the case of stepwise and segment couplings. Applications to assembly of the model decapeptide ACP showed that HDATU was far more effective than HDTU and more effective than HATU under some conditions.

Among a variety of peptide coupling additives which have been studied since 1970, beginning with the classic studies of König and Geiger,[1,2] 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (HODhbt, **1**) proved to be generally superior to all other popular additives in terms of reactivity and coupling efficiency, with the exception of 1-hydroxy-7-azabenzotriazole (HOAt **2**), an additive developed only recently.[3] In addition, the use of HODhbt allows one to follow the progress of the reaction visually by a color change which occurs when acylation is complete.[4,5] Subsequent work on HODhbt, carried out by others,[6−10] has shown that the uronium salt HDTU **3** and combinations such as HODhbt/CIP **4**[11,12] or HODhbt/Fmoc-AA-Pfp esters[13] and, especially, the isolated amino acid esters (Fmoc-AA-ODhbt[4,14,15]) provide many attractive properties for solution- and solid-phase peptide synthesis.



1 **2** **3**



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Although mentioned briefly[16] in the original paper by Harrison and Smith which described the synthesis of HODhbt, 3-hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-traizene (HODhat, **5**) was apparently overlooked by König and Geiger at the time they described the remarkable properties of HODhbt and to our knowledge has never again been cited in the literature. We took up the examination of **5** as a new peptide coupling additive because of its structural similarity to HODhbt and the consideration that the introduction of a nitrogen atom at the 5-position should enhance its reactivity due to the

† Abbreviations: Aib = α-aminoisobutyric acid; ACP = acyl carrier protein decapeptide (65−74); CIP = 2-chloro-1,3-dimethyl-2-imidazolidinium hexafluorophosphate; DCM = dichloromethane; DIC = diisopropylcarbodiimide; DIEA = *N,N*-diisopropylethylamine; EDC = 1-ethyl-3-(3′-(dimethylamino)propyl)carbodiimide; HAPyU = 1-(l-pyrrolidinyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene)-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene)-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU = *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HDTU = *O*-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluromium hexafluorophosphate; Pfp = pentafluorophenyl; Phg = α-phenylglycine; PyBrOP = bromotris(pyrrolidino)phosphonium hexafluorophosphate; PyClU = bis(tetramethylene)-chlorofomamidinium hexafluorophosphate; TEA = triethylamine; TCFH = tetramethylchloroformamidinium hexafluorophosphate; TCM = trichloromethane = chloroform; TFE = trifluoroethanol; TMP = 2,4,6-trimethylpyridine (collidine)

‡ On leave of absence from the Department of Chemistry, Faculty of Science, Alexandria University, Alexandria, Egypt.

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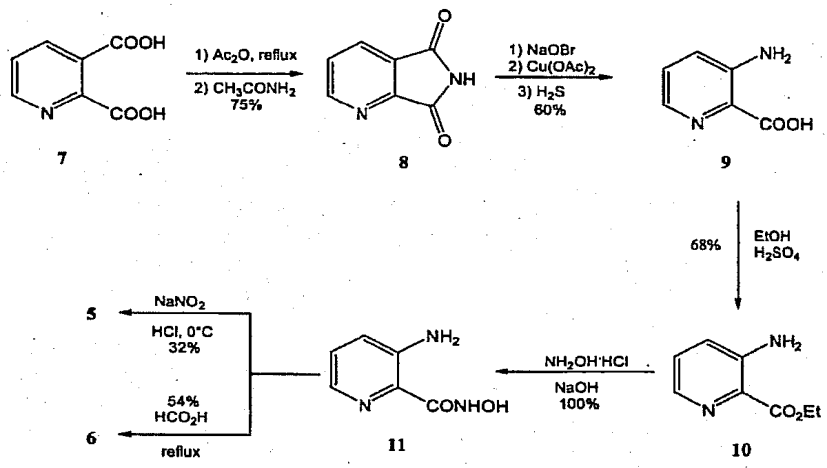
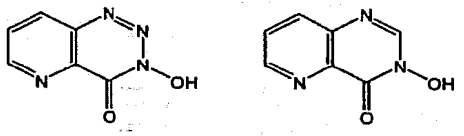
SCHEME 1. Synthesis of HODhat 5 and HODhad 6



electron-withdrawing effect of the pyridine N-atom. Although related to HODhbt in the same way that HOAt is related to 1-hydroxybenzotriazole (HOBt), it should be noted that esters of HODhat are not able to participate in the type of neighboring group effects commonly thought to enhance the effectiveness of HOAt derivatives relative to those of HOBt. Any neighboring group effect occurring in either case would involve the carbonyl function or the adjacent nitrogen atom. The related 3-hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,3-diazene (HODhad, **6**) was also examined.

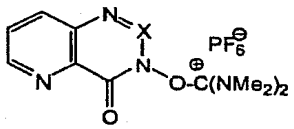


5 **6**

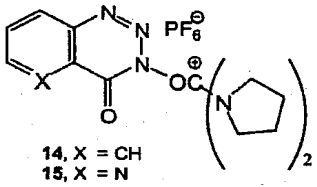
Synthesis of HODhat 5 and HODhad 6. Although the Harrison and Smith paper cited above briefly outlined the preparation of HODhat **5**, no experimental details were given. Syntheses of **5** and **6** are outlined in Scheme 1.[17-19]

To study the reactivity of these compounds, conversion to model active esters, as well as uronium and phosphonium salts, was examined. Peptide coupling additives HOXt (HOAt **2**, HOBt, HODhbt **1**, HODhat **5**, and HODhad **6**) were treated with *N*-benzyloxycarbonyl α-aminoisobutyric acid (Z-Aib-OH) in the presence of EDC·HCl to give in good yield the active esters Z-Aib-OAt, Z-Aib-OBt, Z-Aib-ODhbt, Z-Aib-ODhat, and Z-Aib-ODhad. Carpino and El-Faham[20] had previously recorded the synthesis of Z-Aib-OAt and Z-Aib-OBt from Z-Aib-F. Pivaloyl esters Me_3CCO-OAt, Me_3CCO-OBt, Me_3CCO-ODhbt, Me_3CCO-ODhat, and Me_3CCO-ODhad were synthesized similarly by reaction of pivaloyl chloride with HOXt in the presence of TEA. Characterization data for both types of esters are given in Table 1 in the Supporting Information.

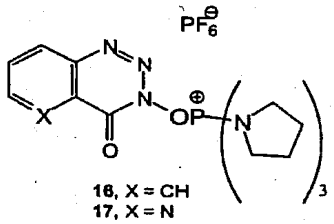
By methods analogous to those used for HODhbt,[7,21] the uronium reagents *O*-(3,4-dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HDATU, **12**) and *O*-(3,4-dihydro-4-oxo-5-azabenzo-1,3-diazin-3-yl)-1,1,3,3-tetramethyluroniumn hexafluorophosphate (HDADU, **13**) and the pyrrolidine analogues *O*-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate (HDPyU, **14**) and *O*-(3,4-dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate(HDAPyU, **15**) were obtained.



12, X = N
13, X = CH



14, X = CH
15, X = N



16, X = CH
17, X = N

Phosphonium salts [(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)oxy]tris(pyrrolidino)phosphonium hexafluorophosphate (PyDOP, **16**[22]) and [3,4-dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)oxy]tris(pyrrolidino)phosphonium hexafluorophosphate (PyDAOP, **17**) were prepared similarly.

The use of isolated Dhbt esters of Fmoc amino acids has previously been described as being advantageous for

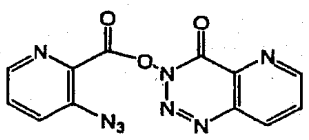
(17) Sucharda, E. *Chem. Ber.* **1925**, *58*, 1727.
(18) (a) Nakadate, M.; Takano, Y.; Hirayama, T.; Sakaizana, S.; Hirano, T.; Okamoto, K.; Hirano, K.; Kawamura, T.; Kimura, M. *Bull. Chem. Pharm. Jpn.* **1965**, *13*, 113. (b) Oakes, V.; Pascoe, R.; Rydon, H. N. *J. Chem. Soc.* **1956**, 1045.
(19) Harrison, D.; Smith, A. C. B. *J. Chem. Soc.* **1959**, 3157.
(20) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695.
(21) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.
(22) Hoeg-Jensen, T.; Olsen, C. E.; Holm, A. *J. Org. Chem.* **1994**, *59*, 1257.

TABLE 2. Approximate Halftimes for Disappearance of Z-Aib-OXt in $CDCl_3$ in the Presence of *p*-Chloroaniline

Z-Aib-OXt	$t_{1/2}$ (min)
Z-Aib-ODhat	8–9
Z-Aib-OAt	9–10
Z-Aib-ODhbt	12–13
Z-Aib-ODhad	70
Z-Aib-OBt	210

peptide synthesis.[4,14,15,23–25] Because of the higher reactivity expected for the corresponding HODhat esters, these compounds were also investigated in the present work. As an example, Fmoc-Ile-ODhat was prepared in good yield and purity by refluxing HODhat with Fmoc isoleucine and thionyl chloride.[15] During its preparation, no azido side product was formed. The method is easy, fast, and efficient and should be well suited for the synthesis of other ODhat esters of Fmoc amino acids.

As described by König and Geiger,[2] during HODhbt/DCC-mediated peptide couplings, side products may be formed due to a competing ring-opening reaction, whereby 2 mol of HODhbt and 1 mol of DCC react to give the *o*-azidobenzoyl active ester, which can then react with a free amino residue during peptide assembly, leading to chain termination. A similar side reaction occurred in the case of HODhat by reaction with DCC or EDC·HCl. In both cases, 3-(3′-azidopicolinoyloxy)-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazine **18** was obtained.



18

During peptide coupling via uronium or phosphonium salts, the N-protected carboxylic acid first reacts with the coupling reagent to give an active ester, which then reacts with the amino component to give the corresponding amide. The latter step is rate-limiting and responsible for loss of configuration. The inherent reactivity of the active ester intermediates is a critical aspect of the value of particular uronium or phosphonium salts.

As a first model, reactions of the hindered active esters Z-Aib-OXt with *p*-chloroaniline (PCA) were studied in $CDCl_3$. Approximate halftimes were determined by proton NMR analysis according to disappearance of the benzylic CH_2 unit (δ 5.2) of the active esters and appearance of the benzylic CH_2 residue (δ 5.5) of product **19**. Results are collected in Table 2.

Z-Aib-PCA Me$_3$CCONR(R′)

19 **20a**, R = H, R′ = Bn
20b, R = Me, R′ = Bn

TABLE 3. Approximate Halftimes for Disappearance of $Me_3CCOOXt$ in $CDCl_3$

$Me_3CCOOXt$	$t_{1/2}$ (min) ($PhCH_2NH_2$)	$t_{1/2}$ ($PhCH_2NHMe$)
$Me_3CCOODhat$	<1	<2 min
$Me_3CCOOAt$	<1	7–8 min
$Me_3CCOODhbt$	<1	18–20 min
$Me_3CCOODhad$	<1	35–40 min
$Me_3CCOOBt$	<1	4.5 h

TABLE 4. Approximate Halftimes for the Disappearance of [Z-Aib-OXt] in Various Solvent Systems in the Presence of *p*-Chloroaniline

coupling reagent	$t_{1/2}$ ($CDCl_3$)	$t_{1/2}$ (CD_3CN)	$t_{1/2}$ (DMF)	$t_{1/2}$ (DMF/$CDCl_3$)[a]
HDATU, **12**	<3 min	70–75 min	2–2.5 hr	30–40 min
HATU[b]	18–24 min	90–95 min	75–85 min	40–45 min
HDTU, **3**	20–25 min	4.5–5 h	6–7 h	2.5–3 h
HBTU[b]	3.5–4 h		10–11 h[c]	

[a] 1:1 mixture of DMF/$CDCl_3$. [b] See list of abbreviations not defined in text. [c] In this case, the halftime is determined by the disappearance of acid *Z*-Aib-OH and appearance of both intermediate active ester and amide **19**.

It was found that the ODhat ester is slightly more reactive even then the OAt ester, which was previously found to be the most reactive derivative among these esters. Interestingly, despite the structural similarity between HODhat **5** and HODhad **6**, the reactivities of the corresponding active esters are very different. This may be due to the presence or absence of additional neighboring group effects promoted by the presence or absence of a nitrogen atom substituted at the 2-position. On the other hand, comparison of the OBt and ODhad esters demonstrates the importance of the neighboring carbonyl group.

As a second model to test the reactivity of these active esters, the pivaloyl esters were treated with benzylamine and *N*-methylbenzylamine, which led to the formation of amides **20a** and **20b**, respectively. Approximate halftimes for these reactions were determined by proton NMR analysis, according to the disappearance of the methyl peak (δ 1.5) for pivaloyl-OXt and the appearance of the methyl peak for products **20a** (δ 1.2) or **20b** (δ 1.3). Results are collected in Table 3.

In the case of benzylamine, all reactions were rapid whereas in the case of the more hindered *N*-methyl derivative, clear reactivity differences were seen according to the following order: ODhat> OAt> ODhbt> ODhad> OBt. Again the greater reactivity of the HODhat ester relative to that derived from HOAt is seen.

A related model system used to compare relative rates of coupling processes involves reaction of Z-Aib-OH with *p*-chloroaniline (PCA) in the presence of a coupling agent. Because formation of intermediate Z-Aib-OXt is usually very fast, halftimes are determined by disappearance of the benzylic CH_2 residue (δ 5.2) of the active ester and appearance of the benzylic CH_2 unit (δ 5.05) of the product **19**, unless otherwise noted. Approximate halftimes are collected in Table 4. In this case, various solvent systems were examined.

Interestingly, in all solvent systems examined except for DMF, the new coupling reagent was found to be more reactive than HATU. In $CDCl_3$, HDATU is at least six

(23) Meldal, M.; Holm, C. B.; Bojesen, G.; Jakobsen, M. H.; Holm, A. *Int. J. Pept. Protein Res.* **1993**, *41*, 250.
(24) Reimer, K. B.; Meldal, M.; Kusumoto, S.; Fukase, K.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1993**, 925.
(25) Knapp, D. R.; Oatis, Jr., J. E.; Papac, D. J. *Int. J. Pept. Protein Res.* **1993**, *42*, 259.

TABLE 5. Effect of Coupling Reagent, Base, and Solvent on the Preservation of Configuration during the Formation of 21 via [1 + 1] Coupling

coupling reagent	additive	base (equiv)	solvent	yield (%)	DL (%)
HDATU, **12**		DIEA (2)	DMF	83.9	4.8
HDTU, **3**		DIEA (2)	DMF	78.4	12.8
HATU[a]		DIEA (2)	DMF	71.7	2.8
HBTU[a]		DIEA (2)	DMF	81.3	6.3
HDATU, **12**		TMP (2)	DMF	87.5	6.0
HDTU, **3**		TMP (2)	DMF	80.7	16.0
HATU[a]		TMP (2)	DMF	90.8	3.8
HBTU[a]		TMP (2)	DMF	85.4	8.7
DCC	HODhat (1)	TMP (1)	TFE/TCM[b]	74.8	0.4
DCC	HODhbt (1)	TMP (1)	TFE/TCM[b]	71.8	0.8
DCC	HOAt (1)	TMP (1)	TFE/TCM[b]	69.2	0.3

[a] See list of abbreviations not defined in text. [b] In this case, 1.3 mL of trifluoroethanol–chloroform (1:3 v/v) was used as solvent.

times as reactive as HATU and about eight times as reactive as HDTU. So far, in every case tested HDATU was shown to be significantly more reactive than HDTU.

To test the configuration-retention effectiveness of the new additives HODhat **5** and HODhad **6**, and the new coupling reagents HDATU **12**, HDADU **13**, HDAPyU **15**, HDPyU **14**, PyDAOP **17**, and PyDOP **16**, several previously studied model peptide systems (**21**–**24**) and a system **25** previously studied by Sakakibara[8] were examined. These involve a [1 + 1] stepwise coupling and three [2 + 1] and one [3 + 3] segment couplings.

Z-Phg-Pro-NH_2 **21**

Z-Phe-Val-Pro-NH_2 **22**

Z-Gly-Phe-Pro-NH_2 **23**

Z-Gly-Gly-Val-Ala-Gly-Gly-NH_2 **24**

H-Gly-Leu-Phe-OBzl **25**

For the sensitive coupling of the urethane-protected Z-Phg-OH to H-Pro-NH_2 to give **21**, HDATU was more effective in preserving configuration than HDTU and HBTU, but not better than HATU. Curiously with this system, use of the base diisopropylethylamine (DIEA) proved more satisfactory than collidine (TMP), a result that is rarely observed in the case of the corresponding segment couplings. Results are collected in Table 5 and its more extensive version in the Supporting Information section.

With carbodiimide in the so-called "structure-breaking" combination solvent TFE/TCM recommended as the best solvent for use with HODhbt by Sakakibara, HODhat was even more effective than HODhbt. Thus, EDC/HODhat gave 0.5% of the DL-isomer, whereas EDC/HODhbt led to 1.3% of the same form. For DCC/HODhat and DCC/HODhbt in the presence of 1 equiv of TMP, the figures were 0.4% and 0.8%, respectively.

For the well-studied segment coupling of Z-Phe-Val-OH to H-Pro-NH_2 leading to tripeptide **22**, the 5-aza derivative of HDTU was generally less effective than the parent system. Best results were generally obtained with HATU and other HOAt-derived reagents, except for the case of carbodiimide reagents in the combination solvent TFE/TCM according to the Sakakibara technique. The results are collected in Table 6 and its more extensive version in the Supporting Information section..

For the rather insensitive case of the segment coupling of Z-Gly-Phe-OH– to H-Pro-NH_2 the results paralleled those for **22**. Results are presented in Table 7 in the Supporting Information section. In contrast to the case of **22**, for tripeptide **23** HDATU was similar to or even slightly more effective than HATU.

TABLE 6. Effect of Coupling Reagent, Base, and Solvent on the Preservation of Configuration during the Formation of 22 via [2 + 1] Coupling

coupling reagent	additive	base (equiv)	solvent	yield (%)	LDL (%)
HDATU, **12**		DIEA (2)	DMF	85.4	15.1
HDTU, **3**		DIEA (2)	DMF	81.0	13.3
HDADU, **13**		DIEA (2)	DMF	72.4	27.6
HATU[a]		DIEA (2)	DMF	81.2	12.7
HBTU[a]		DIEA (2)	DMF	89.6	27.4
HDATU, **12**		TMP (2)	DMF	88.8	8.7
HDTU, **3**		TMP (2)	DMF	86.4	8.5
HDADU, **13**		TMP (2)	DMF	83.8	18.6
HATU[a]		TMP (2)	DMF	80.1	5.0
HBTU[a]		TMP (2)	DMF	81.2	14.2
HDATU, **12**	HODhat (1)	TMP (2)	DMF	68.0	7.3
HDTU, **3**	HODhbt (1)	TMP (2)	DMF	65.0	4.0
HATU[a]	HOAt (1)	TMP (2)	DMF	62.7	1.8

[a] See list of abbreviations not defined in text.

TABLE 8. Effect of Coupling Reagent, Base, and Solvent on the Preservation of Configuration during the Formation of 25 via [2 + 1] Coupling

coupling reagent	solvent	yield (%)	LDL (%)
EDC/HODhat	DMF	76.2	0.20.
EDC/HODhbt	DMF	88.	0.25
EDC/HOAt	DMF	90.6	0.33
EDC/HOBt	DMF	77.4	0.43[a]
EDC/HODhat	TFE/TCM[b]	98.6	<0.1
EDC/HODhbt	TFE/TCM[b]	96.2	<0.1
EDC/HOAt	TFE/TCM[b]	98.2	<0.1
EDC/HOBt	TFE/TCM[b]	90.0	0.20[c]

[a] Sakakibara reports[8] under the same conditions 3.6% of the LDL-isomer. [b] Combination solvent trifluoroethanol–chloroform (1:3 v/v) was used. [c] Sakakibara reports[8] under the same conditions 0.5% of the LDL-isomer.

The test tripeptide **25** was prepared according to the procedure of Sakakibara.[8] Thus, coupling of H-Phe-OBzl·TosOH with Boc-Gly-Leu-OH in the presence of EDC/additive (HOXt) in various solvents gave a product Boc-Gly-Leu-Phe-OBzl which was BOC-deblocked via 50% TFA/CH_2Cl_2 to give the crude tripeptide, which was directly analyzed by HPLC.

In the EDC-mediated synthesis of **25** carried out in trifluoroethanol/chloroformn (1:3 v/v), the three additives HODhat, HODhbt, and HOAt were found equally effective with less than 0.1% epimerization being observed. Upon switching to DMF as solvent, differences, although small, could be noted. Results are collected in Table 8.

Following preliminary studies with simple di- and tripeptide models **21**–**23** and **25**, a test hexapeptide **24** was assembled. The coupling of Z-Gly-Gly-Val-OH to H-Ala-Gly-Gly-OMe had previously been shown[20,26] to be a sensitive- test for the nature of both coupling reagent and base. Results for the reaction in DMF, in the presence of collidine, are gathered in Table 9. HDATU was found to be more effective in preventing loss of configuration at valine than HATU and other coupling reagents. Epimerization levels up to 8.2% of the DL-form were noted according to the order: HDATU < HATU < HDTU < HBTU.

(26) Carpino, L. A.; Ionescu, D.; El-Faham, A. *J. Org. Chem.* **1996**, *61*, 2460.

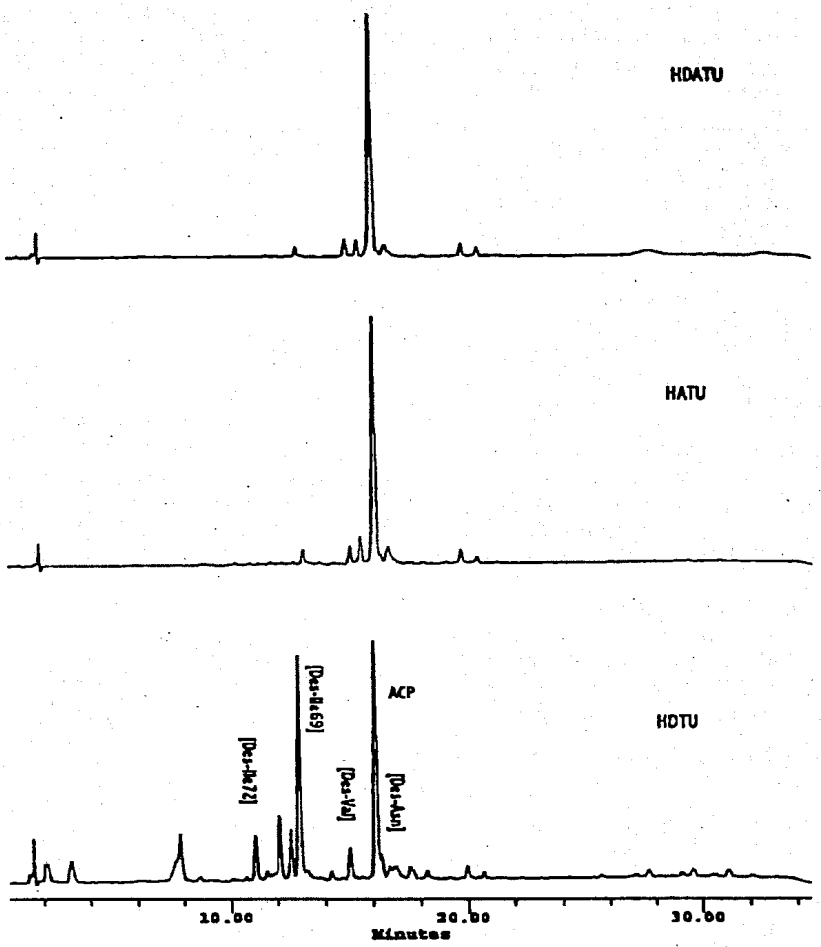


FIGURE 1. Comparison of HPLC traces of ACP decapeptide assembled in methylene chloride via HDATU (ACP 86%), HATU (78%), and HDTU (31%). All syntheses carried out using 4 equiv of the Fmoc amino acid and coupling reagent, 7 min preactivation time, and 3 min coupling time in the presence of 8 equiv of DIEA.

TABLE 9. Effect of Coupling Reagent, Base, and Solvent on the Preservation of Configuration during the Formation of 24 via [3 + 3] Coupling

coupling reagent	base	solvent	yield (%)	LDL (%)
HDATU, **12**	TMP (3)	DMF	98.4	0.8
HDTU, **3**	TMP (3)	DMF	95.0	3.3
HATU[a]	TMP (3)	DMF	96.6	2.4
HBTU[a]	TMP (3)	DMF	85.6	8.2

[a] See list of abbreviations not defined in text.[a]

To demonstrate the suitability of the new HODhat-based coupling reagent HDATU and compare its performance with that of the corresponding guanidinium/uronium analogues HATU and HDTU in solid-phase syntheses, 30 syntheses of the ACP segment H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2 were carried out by an Fmoc/*tert*-butyl protection scheme. Poly(ethylene glycol)-polystyrene (PEG-PS) resin bearing Fmoc-glycine was used as solid support. Peptide elongation was performed manually, coupling times being shortened and excesses of reagents being reduced in order to bring out the differences among the various coupling reagents studied. The methodology has been described previously.[27] Under these conditions, incomplete incorporations were detected for Asn onto Gly, Ile onto Asn, Ile onto Asp, and Val onto Gln. Peptide purity was judged by reverse-phase HPLC analysis, after cleavage from the resin with TFA–H_2O (9:1) for 2 h at room temperature. The results are collected in Table 10 in the Supporting Information.

Analysis of the chromatograms indicated that HDATU is far more effective than HDTU under all conditions examined and more effective even than HATU under some conditions. Methylene chloride was found to be a particularly suitable solvent for HDATU-mediated ACP synthesis. Thus, under so-called "1.5 × 1.5" conditions in DCM, HADTU gave the decapeptide in a putity of 47%, whereas HATU and HDTU led to only 21% and 4% of the desired product, respectively. When a 4-equiv excess of reagents and a 3-min coupling time were used, 86% of ACP was obtained for HDATU, compared with 78% and 31% for HATU and HDTU, respectively (Figure 1).

Although in DMF under "1.5 × 1.5" conditions, the performance of HDATU was not as efficient as HATU

(27) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201.

with or without preactivation, HDATU was found to be the better reagent under normal conditions. Thus, while using a 2 equiv excess of reagents without preactivation for a 5 min coupling, ACP was obtained in 97% purity by HDATU, whereas the corresponding figures were 94 and 81% for HATU and HDTU. With 4-equiv/30 min coupling conditions with a 7-min preactivation time, excellent purity (95%) was obtained for HDATU, whereas with HATU and HDTU, the ACP purity was only 86 and 62%, respectively.

When DIC/HODhat was used as a coupling reagent, satisfactory results were also obtained. Although not suitable under stringent conditions ("1.5 × 1.5"), HODhat could be used as an excellent catalyst and indicator in Fmoc-amino acid pentafluorophenyl (Pfp) ester couplings under normal conditions. A bright yellow-to-orange-red color change was noted which is much clearer than the color change from bright yellow to pale yellow observed with HODhbt. In DMF under conditions involving 3 equiv of Pfp-ester and a 30-min coupling time both HODhat and HODhbt gave the desired ACP product in a purity of over 85%.

For model pentapeptide H-Tyr-Aib-Aib-Phe-Leu-NH_2 **26**,[27,28] which incorporates the highly hindered Aib-Aib unit, whether in DCM or DMF, the new reagent HDATU was not able to equal the results obtained with HATU. For example with 4 equiv of excess acid, 7 min preactivation, and 30 min coupling time, HDATU gave in DMF a peptide of 31% purity, whereas with HATU the purity was 91%.

In conclusion, the long-known but neglected hydroxybenzotriazene derivative HODhat represents a useful, fast-acting coupling additive for both solution- and solid-phase peptide syntheses, with which one can follow the progress of the reaction visually by a color change from bright yellow to orange-red. For stepwise coupling of a urethane-protected amino acid, HDATU was more effective than HDTU, although less effective than HATU. For segment coupling, results were mixed, depending on the system in question. For solid-phase assembly of model peptide ACP, HDATU was shown to be more effective than even HATU under a number of conditions.

Experimental Section

General Methods. Boc-Gly-Leu-OH was prepared by a literature[8] method. TCFH and PyClU were synthesized according to published procedures.

For model peptide H-Gly-Leu-Phe-OBzl **25**,[8] HPLC analysis was carried out on a C-18, 4-μm Waters Novapak column, 3.9 × 150 mm, flow rate 1 mL/min, detection at 220 nm using a linear gradient over 20 min of 0.1% TFA in MeCN and 0.1% aqueous TFA from 1:9 to 11:9. An authentic sample of the DL-isomer was prepared from Boc-Gly-D-Leu-OH and H-Phe-OBzl-TsOH via EDC/HOAt coupling in DMF by following the standard protocol for **25**. Retention times for the LL- and DL-isomers are 17.3 and 17.9 min, respectively, under the conditions specified above.

Other model peptides (Z-Phg-Pro-NH_2 **21**,[29] Z-Phe-Val-Pro-NH_2 **22**,[7] Z-Gly-Phe-Pro-NH_2 **23**,[7] and Z-Gly-Gly-Val-Ala-Gly-Gly-OMe **24**[20,26]) were analyzed according to the procedures previously described. For analysis of pentapeptide H-Tyr-Aib-Aib-Phe-Leu-NH_2 **26** see footnote a of Table 10 (Supporting Information).[27]

Ethyl 3-Aminopicolinate, 10. The procedure given is an improvement over that described previously. A mixture of 3-aminopicolinic acid **9** (5.07 g, 36 mmol), absolute ethanol (20 mL), and concentrated H_2SO_4 (6 mL) was refluxed for 48 h. After cooling, the mixture was concentrated to about 15 mL and poured into 15 g of ice. The mixture was basified with concentrated aqueous ammonia to pH 8–9 with cooling in an ice bath, and the white precipitate that separated was collected by filtration. The filtrate was extracted with ether (4 × 50 mL), and the ether layer washed with brine (4 × 50 mL) and dried over $MgSO_4$. Evaporation of the ether afforded a solid which was treated with decolorizing carbon and recrystallized from benzene-hexane to give 4.05 g (68%) of the ester as white needles: mp 126–127 °C (lit.[18] 131–133 °C, yield 42%); ^{1}H NMR ($CDCl_3$) δ 8.09 (dd, 1),7.23 (dd, 1), 7.04 (dd, 1), 5.76 (s, 2), 4.46 (q, 2), 1.45 (t, 3).

3-Amino-2-picolinehydroxamic Acid, 11. Experimental details were not previously given for this compound. Hydroxylamine hydrochloride (16.3 g, 0.233 mol) was added slowly with stirring and cooling to 110 mL of an aqueous NaOH solution prepared from 18.7 g (0.467 mol) of NaOH. To the solution was added 19.4 g (0.116 mol) of ester **10** portionwise followed by 110 mL of methanol, and the mixture was stirred for 48 h. The solution was concentrated under reduced pressure to about 100 mL and neutralized with cooling to pH 5–6 with 25% HCl. The white precipitate was filtered, washed with a small amount of cold water, and dried over P_2O_5 in vacuo to give 17.8 g (100%) of the acid **11** as a white solid, which was pure enough for the next step. An analytical sample was obtained in 90% yield after two recrystallizations from $MeNO_2$–MeOH–EtOAc as white blocklike crystals: mp 131–133 °C (lit.[19] mp 143–145 °C, yield 49%); ^{1}H NMR(DMSO-d_6/$CDCl_3$) δ 10.91 (s, 1), 8.89 (s, 1), 7.74 (t, 1), 7.16 (d, 2), 6.71 (s, 2); IR (KBr) 3443(m), 3334 (s), 1660 (s, CON), 1606 (s), 1262 (w), 1017 (w), 805 (m) cm^{-1}.

3-Hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazene (HODhat, 5). Experimental details not previously given. To a suspension of finely powdered **11** (7.3 g, 47.6 mmol) in 28 mL of water was added 8.5 mL of concentrated HCl with stirring. While the mixture cooled in an ice bath, a cold solution of $NaNO_2$ (4.93 g, 71.4 mmol) in 5 mL of water was added dropwise and the temperature was maintained below 5 °C. Following addition, stirring in the ice bath was continued for another 30 min, and the solid was filtered, washed with a small amount of cold water, and air dried to give 2.52 g (32%) of the triazene **5** as a yellow solid: mp 195 °C (explodes) [lit.[16] mp 195 °C (explodes)]. The analytical sample was obtained by recrystallization from EtOH–water (9:1 v/v) as light orange-yellow needles: mp 203 °C (explodes); ^{1}H. NMR (DMSO-d_6) δ 9.13 (dd, 1), 8.65 (dd, 1), 8.08 (dd, ^{1}H); IR (KBr) 2600 (broad, OH), 1713 (vs, CON), 1574 (s), 1420 (m), 1230 (sh, s), 1185 (s), 1066 (sh, s), 974 (sh, m), 794 (m) cm^{-1}.

3-Hydroxypicolinic acid (1.6 g, 20%) was also isolated from the mother liquor as light pink needles: mp 222–224 °C. Anal. Calcd for $C_6H_5NO_3$: C, 51.80; H, 3.62; N, 10.07. Found: C, 51.52; H, 3.58; N, 9.98.

3-Hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,3-diazene (HODhad, 6). A mixture of 1.224 g (8 mmol) of hydroxamic acid **11** and 3 mL of 98% formic acid was heated under reflux for 15 min, after which time 8 mL of water was added and the whole mixture was boiled for 15 min and cooled to rt. The precipitate was collected by filtration and washed with water (2 × 5 mL). After being boiled with ethanol twice, 0.71 g (55%) of **6** was obtained, which in an analytically pure form was a yellow solid: mp 318.5–320 °C dec; ^{1}H NMR (DMSO-d_6) δ 12.15 (br, 1), 8.85 (dd, 1), 8.69 (s, 1), 8.17 (dd, 1) 7.84 (dd, 1); IR (KBr) 2625 (broad, OH), 1683 (sh, vs, CON), 1600 (w), 1446 (m), 1410 (s), 1359 (s), 1223 (s), 990 (s), 902 (m), 791 (s) cm^{-1}. Anal. Calcd for $C_7H_5N_3O_2$: C, 51.53; H, 3.09; N, 25.76. Found: C, 51.46; H, 3.00; N, 25.80.

(28) Albericio, F.; Cases, M.; Alsina, J.; Triolo, S. A.; Carpino, L. A.; Kates, S. A. *Tetrahedron Lett.* **1997**, *38*, 4853.

(29) Wenschuh, H.; Beyermann, M.; Haber, H.; Seydel, J. K.; Krause, E.; Bienert, M.; Carpino, L. A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 405.

Z-Aib-ODhat. In an ice bath, 0.3168 g of EDC·HCl (1.65 mmol) was added with stirring to a suspension of Z-Aib-OH (0.3555 g; 1.5 mmol) and HODhat **5** (0.246 g, 1.5 mmol) in 10 mL of THF and 5 mL of DMF. The resulting mixture was stirred at rt for 3 h. Solvents were removed in vacuo, and the oily residue was extracted with 40 mL of EtOAc. The EtOAc solution was washed with 5% aqueous citric acid (3 × 10 mL), 10% $NaHCO_3$ solution (3 × 10 mL), and brine (3 × 10 mL) and dried over $MgSO_4$. Evaporation of the solvent gave a yellow oily residue, which solidified after drying in vacuo over P_2O_5 overnight. The crude solid was purified by flash chromatography with EtOAc as eluent to give 0.46 g (80%) of the ester as a cream yellow solid. For characterization data see Table 1, Supporting Information. Other Aib esters were made similarly.

$Me_3CCOODhat$. Under an atmosphere of dry N_2, TEA (0.42 mL, 3 mmol) was added to a suspension of HODhat **5** (0.3282 g, 2 mmol) in 10 mL of dry methylene chloride. The resulting mixture was cooled to 0 °C, and a solution of pivaloyl chloride (0.27 mL, 2.2 mmol) in 5 mL of dry methylene chloride was introduced dropwise with stirring. The stirring was continued for 30 min in an ice bath, and the temperature was allowed to rise to rt. After 4 h, the mixture was diluted with 30 mL of CH_2Cl_2, and the whole mixture was washed with saturated $NaHCO_3$ (3 × 20 mL), brine (2 × 20 mL), and water (2 × 20 mL) and finally dried over anhydrous $MgSO_4$. Evaporation of solvent gave a pale yellow solid, which was recrystallized from EtOAc–hexane to give 0.31 g (61%) of the analytically pure ester as colorless needles. For characterization data see Table 1, Supporting Information. Other pivaloyl esters were made similarly.

***O*-(3,4-Dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)-1,1,3,3-tetramethyluronium Hexafluorophosphate (HDATU, 12).** Under an atmosphere of dry N_2, 0.22 mL (1.65 mmol) of TEA was added to a suspension of HODhat, **5** (0.246 g, 1.5 mmol), in 10 mL of dry CH_2Cl_2. After being stirred for 5 min, the resulting clear light yellow solution was cooled to 0 °C in an ice bath and 0.4209 g (1.5 mmol) of TCFH was introduced protionwise with stirring. The stirring was continued for 30 min in an ice bath and then at rt for 1.5 h. The precipitate was collected by filtration, washed twice with methylene chloride, and recrystallized twice from MeCN–ether to give 0.42 g (69%) of analytically pure hexafluorophosphate **12** as a white solid: mp 152 °C (explodes); ^1H NMR (CD_3CN) δ 9.19 (dd, 1), 8.69 (dd, 1), 8.13 (dd, 1), 3.21 (s, 12); IR (KBr) 1738 (vs), 1702 (vs) cm^{-1}. Anal. Calcd for $C_{11}H_{15}N_6O_2PF_6$: C, 32.36; H, 3.70; N, 20.58. Found: C, 32.14; H, 3.79; N, 20.47.

***O*-(3,4-Dihydro-4-oxo-5-azabenzo-1,3-diazin-3-yl)-1,1,3,3-tetramethyluronium Hexafluorophosphate (HDADU, 13).** As described for HDATU **12**, diazine HODhad **6** (0.2447 g, 1.5 mmol) was treated with TCFH (0.4209 g, 1.5 mmol) in 10 ml of dry CH_2Cl_2 in the presence of TEA (0.30 mL, 2.1 mmol) to give 0.55 g (90%) of the hexafluorophosphate **13** as a white solid, which was recrystallized twice from MeCN–ether to give 0.48 g (79%) of analytically pure salt as a white solid: mp 203–205 °C dec; ^1H NMR (CD_3CN) δ 8.91 (dd, 1), 8.64 (s, 1), 8.22 (dd, 1), 7.88 (dd, 1), 3.17 (s, 12); IR (KBr) 1701(vs) cm^{-1}. Anal. Calcd for $C_{12}H_{16}N_5O_2PF_6$: C, 35.38; H, 3.96; N, 17.19. Found: C, 35.51; H, 3.86; N, 17.33.

***O*-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-bis(tetramethylene)uronium Hexafluorophosphate (HDPyU, 14).** To a mixture of 0.8157 g (5 mmol) of HODhbt **1** and 0.70 mL (5 mmol) of TEA in 25 mL of dry CH_2Cl_2 at 0 °C was added 1.6633 g (5 mmol) of PyClU portionwise with stirring under an atmosphere of dry N_2. Stirring was continued for 1 h in an ice bath and then at rt overnight, and 50 mL of ether was added causing an oily material to separate. The mixture was stored at −20 °C for 2 days until the oil solidified. The yellow solid was collected by filtration and washed with ice-cold water (4 × 5 mL) to remove TEA·HCl and the residue recrystallized from MeCN–ether to give 1.1 g (44%) of the uronium salt **14** as cream yellow crystals: mp 124 °C (explodes); ^1H NMR (CD_3CN) δ 8.34 (m, 2), 8.17 (m, 1), 8.00 (m,1), 3.74 (t, 8), 1.97 (m, 8); IR (KBr) 1716 (vs), 1676 (vs) cm^{-1}. Anal. Calcd for $C_{16}H_{20}N_5O_2PF_6$: C, 41.83; H, 4.39; N, 15.25. Found: C, 41.64; H, 4.27; N, 15.16.

***O*-(3,4-Dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-y)-1,1,3,3-bis(tetramethylene) uronium Hexafluorophosphate (HDAPyU, 15).** To a mixture of 0.4923 g (3 mmol) of HODhat **5** and 0.46 mL (3.3 mmol) of TEA in 25 mL of dry CH_2Cl_2 at 0 °C was added 1.0 g (3 mmol) of PyClU portionwise with stirring under an atmosphere of dry N_2. Stirring was continued for 1 h in an ice bath and then at rt overnight. The clear light yellow mixture was diluted with CH_2Cl_2 to 50 mL, washed with ice-cold water (2 × 15 mL), and dried over $MgSO_4$. The solvent was removed, the oily residue was dissolved in 5 mL of MeCN to which 30 mL of ether was added, and the whole mixture was stored at −20 °C for several days until the oil solidified. The solid was collected by filtration and redissolved in 20 mL of CH_2Cl_2, and the solution was washed with ice-cold water (2 × 5 ml) and dried over $MgSO_4$. Removal of the solvent gave a pink-yellow solid which was recrystallized from MeCN–ether to give 0.42 g (30%) of the uronium salt **15** as off-white crystals: mp 136.5 °C (explodes); ^1H NMR (CD_3CN) δ 9.17 (dd, 1), 8.67 (dd, 1), 8.11 (dd, 1), 3.75 (t, 8), 1.97 (m, 8); IR (KBr) 1734 (vs), 1679 (vs) cm^{-1}. Anal. Calcd for $C_{15}H_{19}N_6O_2PF_6$: C, 39.13; H, 4.16; N, 18.26. Found: C, 38.94; H, 4.08; N, 18.30.

[1-(3,4-Dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)oxy]-tris(pyrrolidino)phosphoninm Hexafluorophosphate (PyDAOP, 17). To a mixture of 0.2462 g (1.5 mmol) of HODhat **5** and 0.24 mL (1.65 mmol) of TEA in 10 mL of dry CH_2Cl_2 at 0 °C was added 0.6993 g (1.5 mmol) of PyBrOP portionwise with stirring under an atmosphere of dry N_2. Stirring was continued for 1 h in an ice bath and then at rt overnight. The clear light yellow mixture was diluted with CH_2Cl_2 to 25 mL, and the solution was washed with ice-cold water (2 × 10 mL) and dried over $MgSO_4$. The resulting clear light yellow solution was treated with 50 mL of ether, and the solid which separated was collected by filtration to give 0.45 g (54%) of analytically pure phosphoniumn salt **17** as a white solid after recrystallization from MeCN–ether: mp 149 °C dec; ^1H NMR (CD_3CN) δ 9.20 (dd, 1), 8.67 (dd, 1), 8.13 (dd, 1), 3.42 (td, 12), 1.96 (td, 8); IR (KBr) 1742 (vs), 1566 (m), 1462 (sh, w) cm^{-1}. Anal. Calcd for $C_{18}H_{27}N_7O_2P_2F_6$: C, 39.34; H, 4.95; N, 17.84. Found: C, 39.36; H, 5.09; N, 17.90.

Synthesis of Fmoc-Ile-ODhat. Method A.[15] Under an atmosphere of dry N_2, a suspension of Fmoc-Ile-OH (0.3534 g, 1 mmol), HODhat **5** (0.1805 g, 1.1 mmol), and $SOCl_2$ (0.73 mL, 10 mmol) in 8 mL of dry CH_2Cl_2 was refluxed overnight. Evaporation of CH_2Cl_2 and the excess of $SOCl_2$ gave a yellow solid which was purified by flash chromatography through a short silica gel column with a mixture of EtOAc–CH_2Cl_2 (1:1 v/v) as eluent to give, after two recrystallizations from CH_2Cl_2–benzene–ether–hexane, 0.42 g (81%) of the analytically pure ester as a white solid: mp 160.5–162 °C; ^1H NMR ($CDCl_3$) δ 9.15 (dd, 1), 8.58 (dd, 1), 7.96 (dd, 1); 7.76 (dd,2), 7.61 (dd, 2), 7.27–7.44 (m, 4), 5.20 (d, 1), 4.88 (q, 1), 4.49 (d, 2), 4.26 (t, 1), 2.21 (m, 1), 1.70 (m, 1), 1.34 (m, 1), 1.15 (d,3), 1.05 (t, 3); IR (KBr) 1811 (s, COO), 1738 (vs, CONN), 1692 (vs, NHCO) cm^{-1}. Anal. Calcd for $C_{27}H_{25}N_5O_5$: C, 64.91; H, 5.04; N, 14.02. Found: C, 64.77; H, 5.23; N, 13.94.

Method B. Under an atmosphere of dry N_2, 0.1854 g (0.5 mmol) of Fmoc-Ile-Cl was added with stirring to a solution of HODhat **5** (0.0821 g, 0.5 nmol) and DIEA (95.8 μL, 0.55 mmol) in 10 mL of CH_2Cl_2 at 0 °C. Stirring was continued at 0 °C for 30 min and then at rt for 5 h. The resulting light yellow solution was diluted to 30 mL with CH_2Cl_2 and washed quickly with ice-cold brine (2 × 15 mL). After drying over $MgSO_4$ and removing the solvent, the light yellow sticky solid was recrystallized twice from CH_2Cl_2 ether–hexane to give the analytically pure ester as a white solid: mp 161–162 °C. NMR and IR spectra were identical with those of the sample obtained by method A.

Ring-Opening Reaction: Synthesis of 3-(3′-Azidopicolinoyloxy)-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazine 18. To a solution of HODhat **5** (0.3282 g, 2 mmol) in 5 mL of DMF was added 0.2063 g (1 mmol) of DCC portionwise, and the resulting mixture was stirred at rt overnight. The side product DCU was removed by filtration, and the filtrate was evaporated to dryness. The residual solid was recrystallized from $MeNO_2$–EtOAc–hexane to give 0.22 g (65%) of analytically pure **18** as cream yellow needles: mp 153 °C dec; ^{1}H NMR (DMSO-d_6/$CDCl_3$) δ 9.13 (dd, 1), 8.64 (dd, 1) 8.40 (dd, 1), 8.06 (dd, 1), 7.85 (dd, 1), 7.60 (dd, 1); IR (KBr) 1796 (vs, COO), 1733 (vs). Anal. Calcd for $C_{12}H_6N_8O_3$: C, 46.45; H, 1.95; N, 36.12. Found: C, 46.18; H, 1.97; N, 36.28.

Reactivity of HODhat Derivatives.

Z-Aib-OXt Esters. The reaction of Z-Aib-ODhat with PCA is taken as an example to demonstrate the standard method used in order to follow aminolysis via an NMR protocol: To a solution of 47.9 mg (0.125 mmol) of Z-Aib-ODhat in 0.5 mL of $CDCl_3$ was added 15.6 mg (0.125 mmol) of *p*-chloroaniline (PCA). The mixture was immediately transferred to an NMR tube, which was placed in the probe of a Hitachi R-1200 (60 MHz) instrument. Integration of the ^{1}H NMR peaks at δ 1.7 (CH_3 residue of ester Z-Aib-ODhat) and 1.57 (CH_3 residue of amide **19**) [or peaks at δ 5.2 (benzylic CH_2 unit of ester Z-Aib-ODhat) and 5.05 (benzylic CH_2 unit of amide **19**)] as the reaction progressed at the NMR probe temperature (~37 °C) allowed for rough determination of the relative rates. The results given in Table 2 are the average of at least two runs.

Me_3CCOOXt Esters. As in the case with Z-Aib esters, the reaction of pivaloyl ester with *N*-methylbenzylamine is taken here as an example to demonstrate the methodology used: to a solution of 31.0 mg (0.125 mmol) of Me_3CCOODhat in 0.5 mL of $CDCl_3$ was added 15.1 mg (16.1 μL, 0.125 mmol) of $PhCH_2NHMe$. The mixture was immediately transferred to an NMR tube, which was placed in the probe of a Hitachi R-1200 (60 MHz) instrument. Integration of the ^{1}H NMR peaks at δ 1.5 (CH_3 residue of ester Me_3CCOODhat and 1.3 (CH_3 residue of amide **20b**) as the reaction progressed at the NMR probe temperature (~37 °C) allowed for rough determination of the relative rates. The results given in Table 3 are the average of at least two runs.

Model Segment Coupling Reactions. Test couplings were carried out as described previously for Z-Phg-Pro-NH_2, Z-Phe-Val-Pro-NH_2, Z-Gly-Phe-Pro-NH_2, and Z-Gly-Gly-Val-Ala-Gly-Gly-OMe. For Boc-Gly-Leu-Phe-OBzl, 60.6 mg (0.21 mmol) of Boc-Gly-Leu-OH, 85.45 mg (0.20 mmol) of H-Phe OBzl·TsOH, and 0.22 mmol of an appropriate coupling additive (HOXt) were dissolved in 1 mL of DMF or TFE/TCM (1:3 v/v). To the mixture was added a solution of 34.2 mg (0.22 mmol) of EDC in 1 mL of DMF or TFE/TCM, and the whole mixture was stirred at rt overnight. The resulting mixture was diluted with 25 mL of EtOAc, washed with 1 N HCl (2 × 10 mL), 10% $NaHCO_3$ (2 × 10 mL), and brine (2 × 10 mL), and dried over $MgSO_4$. After removal of solvent, the solid was weighed to determine the yield. The solid was then stirred with 2 mL of 50% TFA in a methylene chloride solution for 2 h to deblock the BOC-group. The TFA and CH_2Cl_2 were then removed in vacuo, 20 mL of anhydrous ether was added to the oily residue, and the mixture was stored overnight. The white precipitate which had separated was collected by filtration and washed with ether. About 5 mg of the crude product, containing both LL- and DL-forms of **25**, was dissolved in 4 mL of MeCN and directly analyzed by HPLC using a reversed-phase Waters C_{18} column, with elution by a linear gradient over 20 min of 0.1% TFA in MeCN and 0.1% aqueous TFA from 1:9 to 11:9, at a flow rate of 1.0 mL/min. The retention times for the LL- and DL-forms of **25** were 17.3 and 17.9 min, respectively.

Solid-Phase Assembly of ACP under Stringent Conditions. The standard method previously described according to the so-called "1.5 × 1.5" protocol[27,28] was followed. See Table 10, Supporting Information, and Figure 1.

Acknowledgment. We are indebted to the National Science Foundation (NSF CHE-9003192) and the National Institutes of Health (GM-09706) for support of this work. Millipore, Inc., and Perseptive Biosystems, Inc., kindly supplied the HOAt and HATU used in this study as well as various PEG-PS resins. The National Science Foundation is also thanked for grants used to purchase the high-field NMR spectrometers used in this research.

Supporting Information Available: Table 1, Table 7, and Table 10 and more extensive versions of Tables 5 and 6 and confirming IR and NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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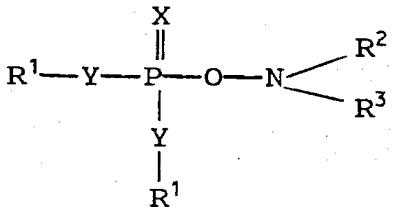
(54) Hardening of gelatin-containing layers.

(57) Gelatin hardeners, suitable for use in hardening gelatin-containing photographic layers, of the formula :



(1)

in which :

X represents O, S or is absent,
each Y independently represents 0, S or a bond,
each R^1 independently represents an aliphatic group of up to 10 carbon atoms or the two R^1 groups together represent the necessary atoms to complete a 5, 6 or 7-membered ring,
$-NR^2R^3$ contains not more than 12 skeletal atoms and
R^2 and R^3 independently represent hydrogen, a cyclic or acyclic group or R^2 and R^3 together represent the necessary atoms to complete a heterocyclic ring.

EP 0 638 842 A1

This invention relates to the hardening of gelatin-containing layers and in particular to the hardening of gelatin-containing photographic layers and to photographic elements containing such layers.

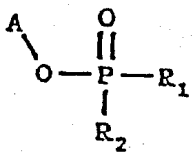
The chemical crosslinking of gelatin is a critical part of producing photographically useful silver halide films. The crosslinking imparts numerous advantages on the coated film through reducing the degree of swelling of the emulsion layer in water, and by increasing the so-called 'melting point' of the gelatin. Improvements in physical characteristics include more resistance to scratching and reduced tendency to become soft or tacky. Suitable hardeners for use in gelatin-containing photographic layers should have no significant detrimental effects on other parameters of the film, e.g., sensitometry.

A number of gelatin crosslinkers are known and find use as hardeners in photographic applications. A commonly used hardener, particularly in photographic elements for Graphic Arts, is formaldehyde. This material is an effective gelatin crosslinker, but has a number of disadvantages which are increasingly of importance, such as the inter-related problems of toxicity and volatility (both in the factory and for the customer) and after-hardening effects. The latter means that it can take some time, often weeks, for the gelatin to reach its final hardness. Since the sensitometric properties of the film depend on film hardness, it is desirable for the hardening process to be complete soon after coating, preferably within one week, for quality control purposes in the manufacturing plant.

Other hardening agents, such as vinyl sulphones, require elaborate multi-step syntheses for their production which increases the cost of the hardening agents. Also some gelatin hardeners can react with other compounds in the photographic construction, for example antihalation dyes and colour couplers making them unacceptable for use in many photographic elements.

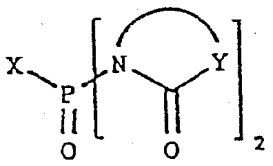
A number of gelatin hardeners containing phosphorus are known.

JP 57044140 discloses pyridinium quaternary salts of substituted aliphatic phosphates:



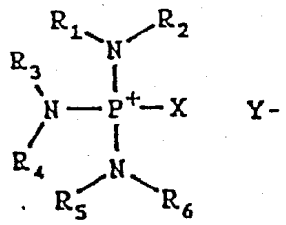
where A is a l-alkyl or l-aryl pyridinium or quinolinium quaternary salt residue.

JP 58-113929 also discloses the use of organophosphorus compounds of the following formula as gelatin hardeners:



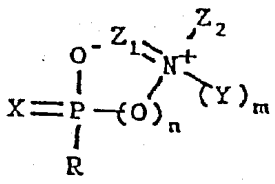
where X is halogen, Y is 0, S, or CH_2:

USP 4668616 discloses the following triaminophosphonium salts as gelatin hardeners:



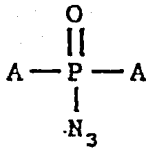
where X is a leaving group to be released on reaction of the compound with a nucleophilic reagent.

USP 5073480 discloses phosphate derivatives of the following formula as quick acting hardeners for proteinaceous gelatin materials:

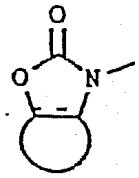


where Z_1 and Z_2 are alkyl, cycloalkyl or aryl, or Z_1 is alkylidene, or Z_1 and Z_2 together form a 5- or 6-membered heterocyclic ring; Y is alkyl, cycloalkyl or aryl; X is O or S; R is alkyl, cycloalkyl, aryl, alkoxy or aryloxy, alkylthio or arylthio, optionally substituted amino or 0^-; n is O or l: m is O or l, m being O if the nitrogen to which Y is attached is involved in a double bond.

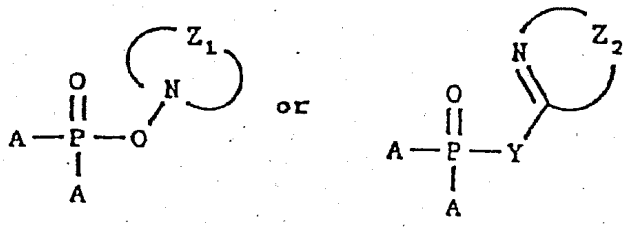
JP 03259241 discloses, as gelatin hardeners, azido phosphorus compounds:



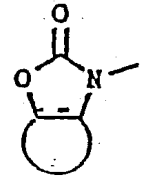
where A is optionally substituted aryloxy or



JP 03259242 discloses, as gelatin hardeners, amino phosphorus compounds:

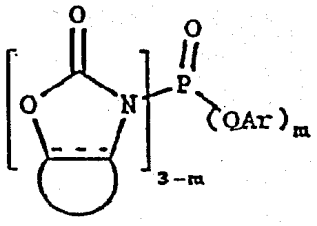


where Y is O or S; Z_1 and Z_2 are the atomic groupings required to complete heterocyclic rings; A is optionally substituted aryloxy or



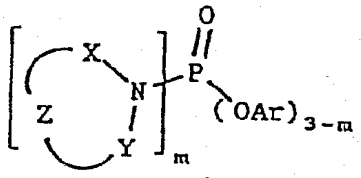
Compounds where A is an aryloxy group are insoluble in water if Z_1 or A does not contain a water-solubilising group. This renders them ineffective as gelatin hardeners.

JP 03259243 discloses, as gelatin hardeners, amino phosphorus compounds of the formula:



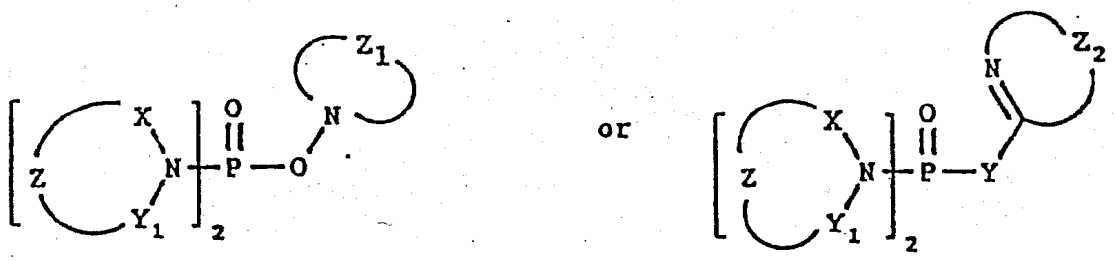
where Ar is optionally substituted aryl; m = 1 or 2.

JP 04157453 discloses, as gelatin hardeners, the following amino phosphorus compounds:



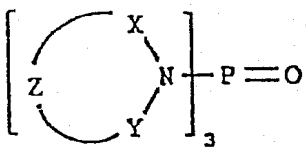
where Ar is optionally substituted aryl; X is CO or SO_2; Y is O, S, N(R), or C(R)=N; Z is the atomic grouping required to complete a 5- or 6-membered heterocyclic ring; m = l or 2.

JP 04157454 discloses similar amino phosphorus compounds:



where X is CO or SO_2; Y_1 is O, S, N(R), or C(R)=N; Y is O or S; Z, Z_1 and Z_2 are the atomic groupings required to complete 5- or 6-membered heterocyclic rings.

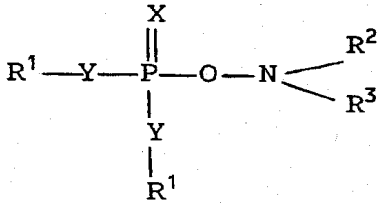
JP 04157455 discloses, as gelatin hardeners, trisubstituted amino phosphorus compounds of the formula:



where X is CO or SO_2; Y is O, S, N(R), or C(R)=N; Z is the atomic grouping required to complete a 5- or 6-membered heterocyclic ring.

The present invention provides an alternative group of phosphorus-containing gelatin hardeners.

According to the present invention there is provided, as a gelatin-hardener, a compound of the formula:



(1)

in which:

X represents O, S or is absent,

each Y independently represents 0, S or a bond,

each R^1 independently represents an aliphatic group or the two R^1 groups together represent the necessary atoms to complete a 5, 6 or 7-member ring and

R^2 and R^3 independently represent hydrogen, a cyclic or acyclic group or R^2 and R^3 together represent the necessary atoms to complete a heterocyclic ring which may optionally have a ring fused thereto.

The present invention presents an inexpensive, readily prepared, soluble, non-volatile gelatin crosslinking agent which solves the problems of vapour toxicity associated with formaldehyde. In addition, hardening of the gelatin has been shown to be essentially complete within three days of coating, particularly if the film is heated immediately after coating. This results in improved aging performance.

The synthesis of the compounds in the present invention is generally straightforward, and usually one step from commercially available materials.

In the compounds of Formula (1) each R^1 independently represents an aliphatic group, generally of up to 10 carbon atoms, preferably up to 5 carbon atoms, such as, an alkyl group, an alkenyl group or an alkynyl group, which may be linear or cyclic. Also included are embodiments in which both R^1 groups together form an alkylene group of 2, 3 or 4 carbon atoms and hence complete a 5, 6 or 7-membered ring.

In the compound of formula (1) each R^1 is preferably an alkyl group of from 1 to 5 carbon atoms.

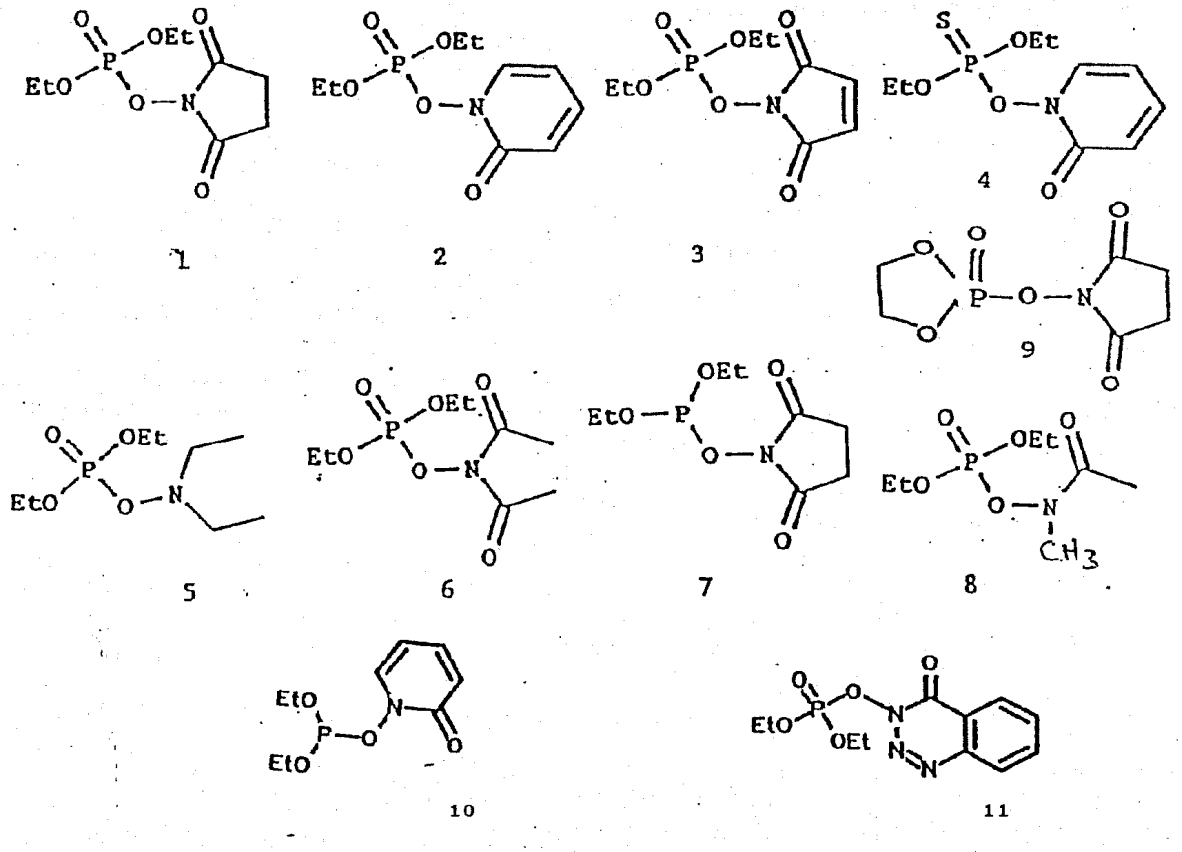
R^2 and R^3 may be independently selected from hydrogen, alkyl, preferably containing 1 to 5 carbon atoms, or acyl, preferably containing up to 5 carbon atoms, e.g., acetyl. Alternatively, and most preferably, R^2 and R^3 together represent the necessary atoms selected from C, N, 0 and S to complete a saturated heterocyclic ring, e.g., succinimide or an unsaturated heterocyclic ring, e.g., maleimide, 2-pyridone etc.

In the compound of Formula (1), the phosphorus atom may be pentavalent (X = 0 or S) or tervalent (X is absent). Preferably, X is 0 or absent.

As is well understood in this technical area, a degree of substitution may be tolerated in R^1 to R^3. As a means of simplifying the discussion and recitation of these groups, the terms "groups" and "moiety" are used to differentiate between chemical species that allow for substitution or which may be substituted and those which do not or may not be so substituted. For example, the phrase "alkyl group" is intended to include not only pure hydrocarbon alkyl chains, such as methyl, ethyl, octyl, cyclo-hexyl, iso-octyl, tertbutyl and the like, but also alkyl chains bearing conventional substituents known in the art, such as hydroxyl, alkoxy, phenyl, halogen (F, C1, Br and I), cyano, nitro, amino etc. The phrase "alkyl moiety" or "alkyl" on the other hand is limited to the inclusion of only pure hydrocarbon alkyl chains, such as methyl, ethyl, propyl, cyclohexyl, iso-octyl, t-butyl and the like.

Generally the group $-NR^2R^3$ contains not more than 12 skeletal atoms.

Examples of compounds useful in this invention include:



Typically, the hardener compounds are incorporated immediately prior to the coating of emulsion or associated gelatin layers. Optimum hardening effect is obtained at substantially neutral pH, e.g., in the range 5 to 7. The hardener compounds are typically added in amounts corresponding to 0.001 mmol to 0.01 mol per gram of gelatin, preferably 0.005 mmol to 0.002 mol per gram of gelatin, most preferably 0.1 mmol to 0.6 mmol per gram of gelatin. The compounds are typically added as a 0.1 - 80 w/w % solution in water or a lower alcohol, preferably as a 1 - 50% solution, most preferably as a 5 - 30% solution, but may also be added neat. After coating, the film may be stored at 10 to 65°C, preferably at 15 to 50°C, and most preferably at 20 to 45°C for a period of up to 100 hours, preferably up to 50 hours, and most preferably up to 24 hours.

The hardness of the film may be measured using any of the published techniques. There is no universally accepted measure of film hardness, but a number of empirical tests have been devised which provide an indication of the relative hardness of a film. One such test is the Dornberg test described below.

Dornberg Test Method

A strip of film of suitable dimensions is soaked in the appropriate developer at a known temperature for a known time. The wet film is then subjected to a point force of increasing torque, which gives a measure of the toughness of the film, which in turn is related to the degree of gelatin crosslinking. The apparatus is described in detail below.

A horizontal bar is attached to a motor drive such that the bar travels at constant speed. Affixed to the bar is a pivot placed 160 mm from a stylus, also attached to the bar, on its lower surface. The stylus consists of 0.014 inch diameter 316 gauge stainless steel wire, looped over a 0.1875 inch diameter steel rod. The stylus is arranged to lie parallel to the direction of travel of the bar. A fixed weight, W, is applied to the upper surface of the rod at a distance from the stylus such that at one extreme the weight is 160 mm from the stylus (i.e., directly above the pivot), and at the other extreme the stylus is 40 mm from the weight. The apparatus is thus designed such that at one extreme the force on the film is zero, and at the other extreme the force corresponds to 0.75W.

As the stylus is drawn across the surface of the film strip, it exerts a force on the film that varies linearly from 0 to 0.75W. The minimum force required to produce a visible scratch is quoted as the Dornberg number, and obviously a higher number indicates a greater degree of hardening. For the most accurate and reproducible results, the weight W is selected so that the scratch covers approximately half the available range. Acceptable Dornberg values are generally at least 5, preferably at least 30, and most preferably at least 50.

The degree of hardness required for a photographic element is largely dictated by the amount of physical handling that the film must survive during conversion and use. Therefore, X-ray films, which are used in cartridges and receive less handling, tend to be softer (Dornberg number < 100, often about 40) than graphic art films (Dornberg number much higher, typically > 800). Of equal importance, however, is the ability to process the film a short time (e.g. 2 hours) after coating. This is critical for quality control purposes in the factory. Elements of the invention are capable of being processed soon after coating (2 hours).

The hardeners of the invention may be used alone or in combination with other hardeners of the invention or known hardeners.

The photographic materials of the invention may comprise any suitable silver halide based imaging material including: colour papers, colour negative films, colour reversal films (either with or without couplers), photosensitive materials for graphic arts (e.g., lith films), photosensitive materials for use with cathode ray tubes or phosphor screens (e.g., x-ray emulsions), photosensitive materials for laser exposure (e.g., imagesetting films), photosensitive materials for dye transfer processes (inhibition transfer processes), photosensitive materials for colour diffusion transfer processes, photosensitive materials for silver salt diffusion transfer processes, photographic emulsion for silver dye-bleach processes, photosensitive materials for thermal development (i.e., photothermographic materials) and photosensitive materials for physical development.

Another major area of utility is in printing plates of the diffusion transfer type, similar to those sold under the trade mark ONYX by Minnesota Mining and Manufacturing Company. Such materials are described, for example, in US Patent No. 4,361,635 and comprise an upper layer of nucleating sites for the physical development of a silver image. When processed in a processing solution comprising a silver halide solvent, silver halide in the unexposed areas dissolves in the processing medium, then diffuses to the surface layer where it contacts the nucleating species and precipitates as metallic silver. The resulting silver image can be used for lithographic printing. Effective hardening of the gelatin is crucial to the run-length of plates of this type, but it must not degrade the hydrophilic character of the non-image areas.

All the above materials generally comprise a support having coated thereon one or more layers of photographic silver halide emulsion, and may additionally comprise one or more additional gelatin layers free from silver halide, such as topcoat layers, interlayers, antihalation layers, etc. The hardeners of the invention may be used in any or all of such layers.

The silver halide photographic emulsion may be any of silver bromide, silver iodobromide, silver iodochlorobromide, silver chlorobromide, silver chloride and silver iodochloride.

The silver halide grains in the photographic emulsion may comprise regular crystals of cubic, orthorhombic, tabular, octahedral or tetrahedral habit, or irregular crystals, such as spherical or composite grains.

Each of the silver halide grains may be made up of a uniform phase through its core and surface layer, or it may be dissimilar in phase between the core and the surface. It is also possible to use two or more independently prepared silver halide emulsions as a mixture. In addition, the silver halide particles may be of the surface latent image type or of the internal latent image type. In the former, the latent image is formed on the surface of the grains, and in the latter, the image is formed inside the grains. The surface latent image type of grain is used for negative-type emulsions and the internal latent image type for internal latent image type emulsions and prefogged direct reversal type emulsions.

As regards the average grain size of the silver halide emulsion, for certain applications, notably graphic arts films and printing plates, fine grains, e.g., 1μm (micrometer) or less, are preferred and very fine grains not larger than 0.5μm are particularly preferable. While the grain size distribution is optional, a monodispersion is preferable for printing plate and graphic art applications. The term "monodispersion" as used herein means that, whether by in weight or number, at least 95% of grains are sized within ± 40% of the mean grain size.

For certain other applications, e.g., X-ray films, a preferred silver halide emulsion comprises laminar grains having a thickness of 0.5μm or less, preferably 0.3μm or less, and a diameter of 0.6μm or greater and in which laminar grains having an average aspect ratio of 5 or more, account for more than 50% of their total projected area.

The silver halide emulsions used in this invention can be prepared according to the processes described, for example, in "Chimie et Physique Photographique" by P. Glafkides (Paul Montel, 1967), "Photographic Emulsion Chemistry" by G.F. Duffin (Focal Press, 1966) and "Making and Coating Photographic Emulsion" by V.L. Zelikman (Focal Press 1964).

When the silver halide grains used in this invention are formed, the growth of grains may be controlled by

adding a silver halide solvent, such as ammonia, potassium thiocyanate, ammonium thiocyanate and thioether compounds, as disclosed in US Patent Nos. 3271157, 3574628, 3704130, 4297439 and 4276374.

The formation or physical ripening of the silver halide crystals may be carried out in the presence of a cadmium salt, a zinc salt, a lead salt, a thallium salt, an iridium salt or complex salt thereof, a rhodium salt or complex salt thereof or a ruthenium salt or complex salt thereof, or mixtures thereof.

The silver halide emulsion may also contain a sensitiser so as to render the emulsion sensitive to any radiation falling within the absorption spectrum of the chosen sensitiser, as described, for example, in Neblette's Handbook of Photography and Reprography pp. 73 to 112 (9th Edition).

Preferred sensitisers include cyanine and merocyanine dyes, the use of which is well known to the person skilled in the art.

The photographic emulsion may also be chemically sensitised. Known methods for chemical sensitisation of silver halide emulsions include sulphur sensitisation, reduction sensitisation and noble metal sensitisation. Chemical sensitisation may be effected by any or a combination of such methods.

The usual method for noble metal sensitisation is gold sensitisation and for this purpose, a gold compound, generally a complex salt of gold, e.g., potassium, chloroaurate, auric trichloride etc. is utilized. Complex salts of other noble metals such as platinum, palladium, rhodium etc., may also be used. Sulphur sensitisers include, in addition to sulphur compounds contained in gelatin, various sulphur compounds such as thiosulphates, thiourea compounds, thiazoles and rhodanines e.g., allyl thiocarbonate, thiourea, allyl isothiocyanate, cysteine etc. Examples of such methods are described in U.S. Patent Specification No. 2,448,060, 2,540,085, 2,597,856, and 2,597,915 and British Patent No. 618961.

Photographic silver halide emulsions useful in the present invention can also be sensitized by other means, such as by alkylene oxide polymers, many of which are well known to those skilled in the photographic art. Typical polyalkylene oxide polymers include those disclosed in U.S. Patent Specification Nos. 2,423,549 and 2,441,389.

The emulsions may also be chemically sensitized with reducing agents such as stannous salts (U.S. Patent Specification No. 2,487,050), polyamines such as diethylene triamine (U.S. Patent Specification No. 2,518,698), polyamines such as spermine (U.S. Patent Specification No. 2,521,925), or bis-(β-aminoethyl) sulfide and its water-soluble salts (U.S. Patent Specification No. 2,521,926).

The emulsions of the invention can also contain speed-increasing compounds of the quaternary ammonium type as disclosed in U.S. Patent Specification Nos. 2,271,623, 2,238,226, 2,334,864, or the thiopolymers disclosed in Canadian Patent Application Nos. 783752 and 783753.

Supersensitisers may also be employed.

The photographic emulsions may be high contrast, e.g., lith films, containing a hydrazine compound or other additives known in the art. Such materials are disclosed, for example, in US Patent Specification Nos. 2322027, 2419974, 2419975, 4166742, 4168977, 4211857, 4224401, 4743739, 4272606, 4272614, 4311781 and 4323643.

The silver halide emulsion may contain a variety of compounds for the prevention of fog that would otherwise occur during the manufacturing process, preservation or photographic processing and for the stabilisation of photographic performance. Examples of such antifoggants and stabilisers include: azoles, such as benzothiazolium salts, nitroimidazoles, nitroindazoles, triazoles, benzotriazoles, benzimidazoles (particularly the nitro-or halogen-substituted benzimidazoles, e.g., bromobenzimidazoles, chlorobenzimidazoles etc.); heterocyclic mercapto compounds, such as mercaptothiazoles, mercaptobenzothiazoles, mercaptobenzimidazoles, mercaptothiadiazoles, mercaptotetrazoles (particularly l-phenyl-5-mercaptotetrazole), and mercaptopyrimidines; thioketo compounds (e.g., oxazolinethione); azaindenes, such as triazaindenes, tetraazaindenes (particularly 4-hydroxy-substituted-(l,3,3a,7)-tetraazaindenes); benzenethiosulphonic acids; benzenethiosulphinic acids and benzenesulphonamide. Amongst these compounds, benzotriazoles (e.g., 5-methylbenzo-triazole and nitroindazoles (e.g., 5-nitroindazole) are preferred. These compounds may also be incorporated in the processing solution.

The photographic materials may also contain other inorganic or organic hardening agents in the photographic emulsion layer or other hydrophilic colloid layer. For this purpose chromium salts (chrome alum, chromium acetate etc.), aldehydes (formaldehyde, glyoxal, glutaraldehyde etc.), N-methylol compounds (dimethylolurea, methyloldimethylhydantoin etc.), dioxane derivatives (2,3-dihydroxydioxane etc.), active vinyl compounds (1,3,5-triacryloyl-hexahydro-s-triazines, 1,3-vinyl-sulphonyl-2-propanol etc.), active halogen compounds (2,4-dichloro-6-hydroxy-s-triazine etc.), mucohalogenic acids (mucochloric acid, mucophenoxychloric acid etc.), and the like may be used.

The silver halide emulsion or other hydrophilic colloid layer may also contain a variety of surface active agents for purposes such as the improvement of coating properties, antistatic properties, slip properties, emulsion dispersibility, anti-adhesion properties and photographic properties (e.g., development acceleration, in-

crease in contrast, sensitisation etc.).

Non-ionic surfactants may be employed such as saponin (steroidal), polyethylene glycol, polyethylene glycol/polypropylene glycol condensate, polyethylene glycol alkyl ethers, polyethylene glycol alkyl aryl ethers, polyethylene glycol esters, polyethylene glycol sorbitan esters, polyalkylene glycol alkylamines or alkylamides, silicone polyethylene oxide adducts), glycidol derivatives (e.g., alkenylsuccinic acid polyglyceride, alkylphenol polyglyceride), polyhydric alcohol-fatty acid esters, sugar alkyl esters etc.

Anionic surfactants containing acid groups, such as a carboxyl group, a sulpho group, a phospho group, a sulphuric acid ester group, a phosphoric acid ester group etc., for example, alkylcarboxylate, alkylsulphonates, alkylbenzenesulphonates, alkylnaphthalenesulphonates, alkylsulphuric acid esters, alkylphosphoric acid esters, n-acyl-n-alkyltaurines, sulphosuccinic acid esters, sulphoalkylpolyoxyethylene alkylphenyl ether, polyoxyethylene alkylphosphoric acid esters etc., may also be used.

Amphoteric surfactants such as amino acids, aminoalkylsulphonic acids, aminoalkylsulphuric or phosphoric acid esters, alkylbetaines, amine oxides etc.; and cationic surfactants, such as alkylamines, aliphatic or aromatic quaternary ammonium salts, heterocyclic quaternary ammonium salts, such as pyridinium salts, imidazolium salts etc., aliphatic or heterocyclic ring-containing phosphonium or sulphonium salts etc. may be used.

The photographic emulsion layer and/or the hydrophilic colloid layer may also include a matting agent, such as silica, magnesium oxide, polymethyl methacrylate etc., for the purpose of preventing adhesion.

The silver halide emulsion may contain a discolouration prevention agent, colour-fog preventing agent, UV light absorber, and other additives. Detailed description of these additives will be found in Research Disclosure Vol. 176 (1978, XII) RD-17643.

In certain embodiments, the emulsion or an associated layer may contain one or more developing agents for silver halide (or their precursors), as described, for example, in Research Disclosure No. 17364 (Sept. 1978), Canadian Patent No. 766,708 and European Patent Application No. 0,532,192. This enables activation processing in alkaline solutions that are essentially free from conventional developers.

The finished emulsion and any associated layers are applied to a support which may be made of an opaque material, such as baryta paper, resin-coated paper, synthetic paper or a transparent material, such as glass or a plastics film, e.g., cellulose triacetate; cellulose diacetate, nitrocellulose, polystyrene, polyethylene terephthalate (polyester) etc.

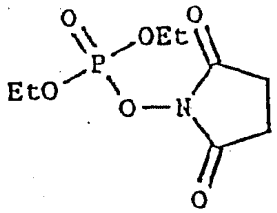
The photographic material of the invention can be exposed using conventional sources, such as natural light (sunlight), tungsten lamps, fluorescent lamps, mercury lamps, xenon arc lamps, carbon arc lamps, xenon flash lamps and CRT spots. The exposure time is not limited to that for ordinary cameras (1/1000 sec to 1 sec) and exposures as short as $1/10^4$ to $1/10^7$ seconds by a xenon flash lamp or laser scanner are also possible. Exposures longer than 1 second are also possible. If necessary, it is possible to control the spectral energy distribution of the light for exposure by means of a proper colour filter. The light-sensitive material of the invention can be exposed with laser light or light emitted by the fluorescent material excited by electron ray, X-ray, γ-ray, or α-ray.

The light-sensitive material of the invention may be processed by any known method with any known processing solution, such as those disclosed in Research Disclosure No. 176, pp. 28-30 (RD-17643). Thus, for example, dihydroxybenzenes (e.g., hydroquinone), 3-pyrazolidones (e.g., 1-phenyl-3-pyrazolidone and 4,4-dimethyl-l-phenyl-3-pyrazolidone), aminophenols (e.g., 4-methylaminophenol) etc., can be used alone or in combination.

This invention will now be described with reference to the following Examples.

Example 1

a) Synthesis of Compound 1



A solution of N-hydroxysuccinimide (25.2 g, 0.219 mol) in dry THF (400 ml) was treated with triethylamine (28.8

ml), whereupon a colourless precipitate formed. Diethyl chlorophosphate (30 ml, 0.20 mol) was added dropwise and the mixture was heated to reflux for 16 hrs, resulting in the formation of a thick white precipitate. After this time no chlorophosphate was present as shown by ^{31}P NMR of the crude reaction mixture. The mixture was allowed to cool, and was then filtered and the filtrates were evaporated to leave an oil. This oil was dissolved in ethyl acetate (200 ml), the resulting solution being washed with saturated aqueous sodium bicarbonate solution (50 ml) and water (100 ml). The aqueous wash was back-extracted with ethyl acetate (100 ml), the combined ethyl acetate extracts being dried over magnesium sulphate. The magnesium sulphate was removed by filtration, and the combined filtrates were evaporated to leave a colourless oil. The last traces of ethyl acetate were removed from this oil by azeotroping with dichloromethane, to leave the product as a colourless oil (46.0 g, 92%).

b) Evaluation of Compound 1 in a Fine-Grained Silver Chlorobromide Emulsion

A fine-grained 0.09 micron, 96% silver chlorobromide emulsion with rhodium doping was prepared and chemically sensitized using a thiosulphate and gold digestion using methods known to those skilled in the art. Samples of this emulsion were coated on to a polyester base material such that the silver coverage was 2.5 gm^{-2}, the total gelatin coverage was 3.6 gm^{-2}, and the hardener, which was added as a 10% solution in methanol, was added at the levels listed in the following Table. Hardness values were determined after 2 hours at room temperature by the Dornberg method.

Hardener mmol/g gel	Dornberg Number
0.13	30
0.18	51
0.23	60
0.12	260*

* measured after heating at 38°C for 16 hours

Thus Compound 1 shows good hardening activity in this emulsion.

c) Effect of Compound 1 on Sensitometric Parameters

Coatings of a fine-grained emulsion, prepared as described above, were exposed on a UV Contacting exposing frame through a 0 - 2.6 continuous tone wedge and processed through 3M RDC V Rapid Access chemistry. The sensitometric parameters for coatings containing Compound 1, and a control hardened with formaldehyde, are shown in the following Table.

	Hardener mmol/g gel	Dmin	Dmax	SP-1*	Con1**
Cpd1	0.13	0.04	4.62	1.82	15.26
HCHO	0.34	0.06	4.59	1.77	16.71

* speed at density 0.1 above fog
** contrast between densities 0.1 and 2.5 above fog

d) Evaluation of Compound 1 in a Scanner Emulsion

A 0.25 micron cubic chlorobromide emulsion (64% AgCl) was prepared by a conventional double-jet precipitation method familiar to those skilled in the art. The emulsion was doped with iridium and ruthenium metal ions to provide good reciprocity behaviour, and was chemically sensitised with sodium thiosulphate and sodium